

# Isolation and characterization of proSS<sub>1-32</sub>, a peptide derived from the N-terminal region of porcine preprosomatostatin

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A peptide derived from the N-terminal region of porcine prosomatostatin, proSS<sub>1-32</sub>, has been purified to homogeneity from extracts of porcine upper intestine. Amino acid analysis revealed that the peptide consists of 32 residues. The complete primary structure was determined as:

APSDPRLRQFLQKSLAAAAGKQELAKYFLAEL

This sequence obviously comprises residues 1–32 of porcine prosomatostatin since it is identical to the corresponding sequence in human preprosomatostatin. The postulated cleavage site in porcine prosomatostatin is a Leu–Leu bond between residues 32 and 33, thus confirming previous studies of the processing of the somatostatin precursor in the rat and transgenic mouse.

<i>N-terminal prosomatostatin peptide</i>	<i>Isolation</i>	<i>Porcine gut extract</i>	<i>Primary structure</i>
<i>Preprosomatostatin processing</i>		<i>Gastrointestinal peptide</i>	

## 1. INTRODUCTION

The structure of preprosomatostatin (preproSS), the precursor of somatostatin-14 (SS-14) and somatostatin-28 (SS-28), has been deduced from nucleotide sequences of anglerfish [1,2], catfish [3–6], rat [7–9] and human [10] cDNAs as well as from a human [11] and rat [12] genomic library. The N-terminal part of prosomatostatin is highly conserved, so that only 2 residues are different between the rat and human precursor. Very little, however, is known about the posttranslational processing of this region of prosomatostatin (proSS). SS-14, SS-28 and its N-terminal fragment, SS-28<sub>1-12</sub>, have been isolated and characterized from mammalian tissues [13–18]. It has been

postulated that several other peptides may derive from the prosomatostatin sequence [19,20].

While characterizing side-fractions obtained during the isolation procedure for vasoactive intestinal peptide (VIP) from porcine gut extracts [21], we identified a peptide with the N-terminal sequence of prosomatostatin. We report here the isolation, characterization and complete primary structure of this peptide derived from porcine prosomatostatin, which we have designated 'proSS<sub>1-32</sub>'.

## 2. MATERIALS AND METHODS

### 2.1. Isolation of the peptide

As starting material a highly purified peptide

fraction from an upper porcine gut extract, the 'post-secretin' fraction, from which VIP has been isolated [21,22], was used. This material was chromatographed on a CM-cellulose column in 20 mM Na-phosphate, pH 6.4, using a salt gradient (0–0.3 M NaCl) for elution (see legend to fig.1). Fractions I–VIII were further purified by high-performance liquid chromatography (HPLC) on a reversed-phase C-18 wide-pore column (Vydac RP 201 TPB) using a Waters HPLC system. Unidentified peaks were characterized after rechromatography by HPLC retention time and UV absorbance at 214 and 280 nm, compared to mixtures of known gastrointestinal peptides, used as reference. Structural information was gained by amino acid and N-terminal sequence analysis.

Somatostatin-like immunoreactivity was measured by radioimmunoassay using an antiserum directed towards the central residues of SS-14 that cross-reacts fully with SS-28 [23].

## 2.2. Structural analysis

Amino acid composition was determined with a Durrum D-500 automatic amino acid analyzer after hydrolysis with 5.7 N HCl containing 0.5% phenol in evacuated tubes at 110°C for 24 or 72 h. For sequence determination the peptide was digested at 37°C with TPCK-trypsin in 0.1 M  $\text{NH}_4\text{HCO}_3$ , pH 8.2, for 4 h or with *Staphylococcus aureus* V8 protease in the same buffer, but at pH 7.8, for 48 h. The cleavage products were separated by HPLC. The primary structure was determined by manual DABITC-Edman degradation (dimethylaminoazobenzene isothiocyanate method) [24], liquid-phase sequencing on a Beckman model 890 [25] or gas-phase sequencing on an Applied Biosystems model 470A [26]. The phenylhydantoin derivatives were identified by HPLC [27].

## 3. RESULTS

### 3.1. Isolation of the peptide

The initial cation-exchange chromatography of the starting material ('post-secretin' fraction) on CM-cellulose in 20 mM Na-phosphate, pH 6.4, is shown in fig.1. Peptides were eluted by an NaCl gradient (0–0.3 M). Fractions I–VIII were further separated by reversed-phase HPLC on a Vydac

C-18 column in a trifluoroacetic acid (TFA)/acetonitrile solvent system as described in the legend to fig.2, which shows the HPLC absorbance profile of fraction II.

Retention time analysis, UV absorbance ratio at 214 and 280 nm (not shown) and screening N-terminal sequence determination of the first 6 residues by manual DABITC-Edman degradation revealed in this fraction the presence of several peptides with structural and chromatographic properties clearly different from those of previous-

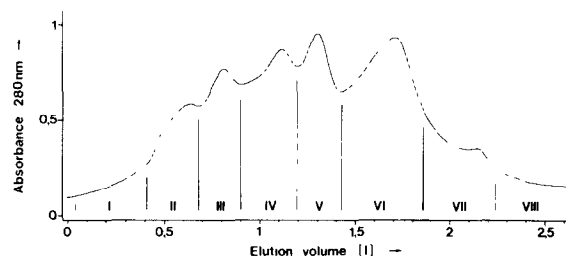


Fig.1. Cation-exchange chromatography of the starting material (post-secretin fraction) on CM-cellulose in 20 mM Na-phosphate, pH 6.4, using a NaCl gradient (0–0.3 M) for elution. Fractions I–VIII were further characterized by HPLC.

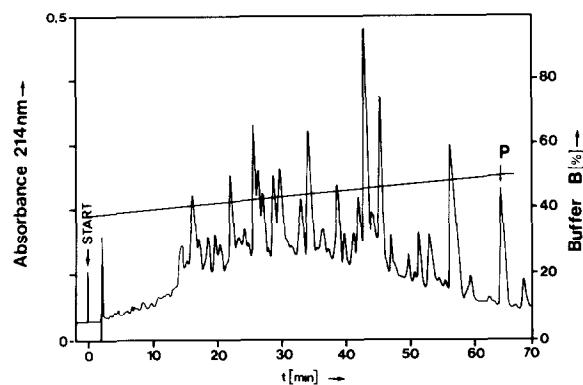


Fig.2. HPLC elution profile of fraction II from the CM-cellulose chromatography (fig.1). 0.8 mg were applied to a Vydac RP 201 TPB reversed-phase wide-pore C-18 column (10  $\mu\text{m}$ , 300 Å, 0.46  $\times$  25 cm) and eluted at 40°C with a linear gradient from 40 to 50% solvent B in 65 min. Flow rate, 1.5 ml/min; solvent A, 0.1% TFA; solvent B, 0.12% TFA in 70% acetonitrile. Peaks were characterized by retention time analysis, UV absorbance at 214 and 280 nm (not shown) and N-terminal sequence determination. The peak marked P contained peptide proSS<sub>1–32</sub>.

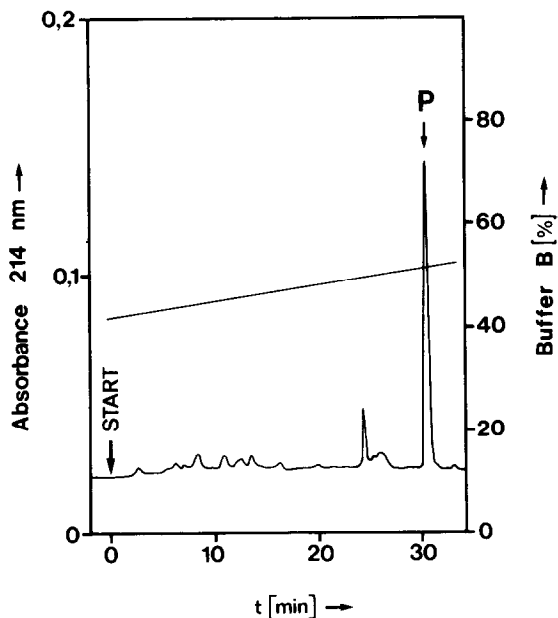


Fig.3. HPLC rechromatography of peak P from fig.2 using a different linear gradient; other conditions were as described in fig.2. Peptide proSS<sub>1-32</sub> eluted in essentially pure form.

ly isolated gastrointestinal peptides used as reference. The peak marked 'P' contained a peptide with the N-terminal sequence APSDPR which is identical with the N-terminus of pro-

somatostatin. HPLC rechromatography of this peak in the TFA/acetonitrile system under the conditions detailed in the legend to fig.3 revealed the peptide in essentially pure form. It did not react with an antiserum directed against the SS-14 sequence, thus indicating that the peptide was not identical with the complete prosomatostatin precursor, proSS<sub>1-92</sub>.

### 3.2. Structural analysis

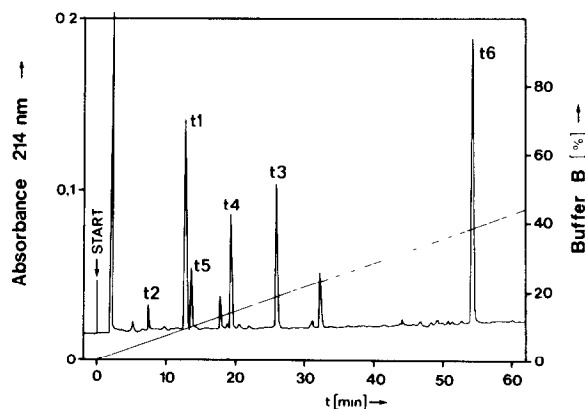
Table 1 shows the amino acid analysis of the peptide. It comprises 32 residues. Tryptic cleavage resulted in 6 fragments (t1-t6) which were separated by HPLC as shown in fig.4, using the same chromatographic conditions as described in the legend to fig.2. By cleavage with *S. aureus* V8 protease, 2 fragments were generated and complete separation was also achieved by HPLC (not shown). Table 1 shows the amino acid compositions of the enzymatic fragments. The intact peptide which we named proSS<sub>1-32</sub> and fragment SV8-1 comprising 23 residues were degraded sequentially up to position 20 by the manual DABITC-Edman method and by liquid-phase sequencing. The primary structure of peptide t6 was established by gas-phase sequencing, all other enzymatic fragments were completely sequenced manually. Fig.5 shows the amino acid sequence of proSS<sub>1-32</sub>.

Table 1

Amino acid compositions of proSS<sub>1-32</sub> and its peptide fragments generated by cleavage with trypsin and *S. aureus* V8 protease

	proSS <sub>1-32</sub>	t1	t2	t3	t4	t5	t6	SV8-1	SV8-2
Asp	1.1 (1)	1.1 (1)						1.1 (1)	
Ser	2.2 (2)	1.0 (1)			1.0 (1)			2.0 (2)	
Glu	5.6 (5)			2.2 (2)		2.0 (2)	1.3 (1)	4.3 (4)	1.0 (1)
Pro	2.2 (2)	2.0 (2)						1.9 (2)	
Gly	1.2 (1)				1.1 (1)			1.4 (1)	
Ala	6.8 (7)	1.0 (1)			3.6 (4)	1.0 (1)	1.2 (1)	4.6 (5)	2.0 (2)
Leu	6.6 (6)		1.0 (1)	1.0 (1)	1.2 (1)	1.0 (1)	2.5 (2)	2.9 (3)	2.6 (3) <sup>a</sup>
Tyr	0.9 (1)						0.9 (1)		0.8 (1)
Phe	1.8 (2)			0.9 (1)			1.0 (1)	1.0 (1)	0.9 (1)
Lys	3.4 (3)			1.0 (1)	1.1 (1)	1.1 (1)		1.9 (2)	1.0 (1)
Arg	1.8 (2)	1.1 (1)	1.0 (1)					1.8 (2)	
Sum	32	6	2	5	8	5	6	23	9

<sup>a</sup> After hydrolysis for 24 and 72 h



**Fig.4. Separation of the tryptic peptides of proSS<sub>1-32</sub> by HPLC using the same chromatographic conditions as described in fig.2. Peptides t1-t6 eluted in pure form.**

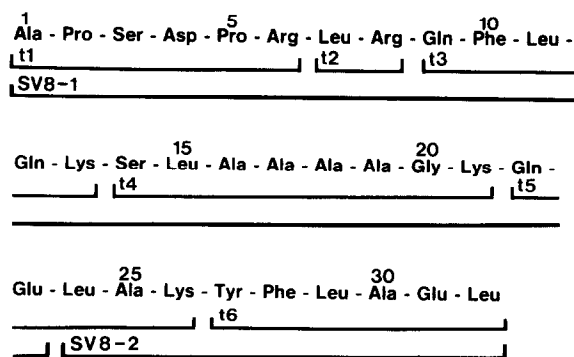
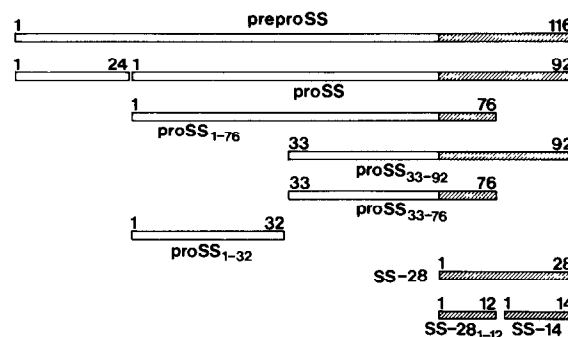


Fig.5. The primary structure of peptide proSS<sub>1-32</sub> and its enzymatic fragments t1-t6, SV8-1 and SV8-2.

## 4. DISCUSSION

The isolation and complete characterization of proSS<sub>1-32</sub>, as shown in fig.6, derived from the N-terminal part of porcine prosomatostatin, confirm the observations made by Goodman et al. [8] and Benoit et al. [20] concerning the cleavage site of the signal peptide in the human and rat precursor, respectively. In pig, Ala in position 25 of preproSS constitutes the N-terminus of proSS. Porcine proSS<sub>1-32</sub> is identical to the corresponding sequence in human preproSS, whereas in the rat precursor position 19 is Thr for Ala. The post-translational processing of preprosomatostatin leads to the preferential formation of biologically active SS-28 in the gut, but to SS-14 in brain, pan-



**Fig.6.** Schematic synopsis of preprosomatostatin (preproSS) and the proposed or isolated peptides derived from it; numbers refer to N- or C-terminal amino acid residues adjacent to possible cleavage sites. In preproSS, residues 1–24 represent the signal peptide.

creas and antrum. Release of SS-28<sub>1-12</sub>, the N-terminal part of SS-28, has also been demonstrated [28]. Despite the high structural conservation of the N-terminal pro-sequence between the rat and human precursor, its processing and biological function remains unclear. Studies by Benoit et al. [19,20] indicate that in addition to the well-characterized cleavage sites of preproSS between position 24–25, 88–89 and 102–103, generating proSS, SS-28 and SS-14, respectively, a fourth processing site at the Leu–Leu bond (position 56–57), corresponding to position 32–33 of proSS, may exist. Cleavage at this site produces a 5 kDa fragment, proSS<sub>33–76</sub>, which contains the SS-28<sub>1–12</sub> sequence as C-terminus (see fig.6). In fact, a peptide was isolated from rat brain extracts with N-terminal LSEPNQ, corresponding to position 33–38 of proSS [20]. A similar 5–6 kDa peptide containing the SS-14 sequence has been demonstrated to be present in a human pheochromocytoma and in normal human intestine [29,30].

The existence in porcine gut extracts of proSS<sub>1-32</sub>, being the N-terminal counterpart of the 5 kDa rat brain peptide (fig.6), supports the hypothesis that cleavage of the Leu-Leu bond in position 32-33 may represent an important step in the processing of proSS. This assumption is in good agreement with recent studies of the post-translational processing of preproSS in transgenic mice [31] suggesting that a 5-6 kDa peptide is generated in some tissues, possibly by cleavage

within this sequence region. Although being uncommon, a Leu-Leu cleavage is also involved in the formation of angiotensin I [32]. However, it cannot be ruled out that cleavage at this site in proSS may represent, at least in part, an artifact produced by the extraction method, although different extraction and chromatography procedures were used in the present and previous studies [20,29,31].

Recently, it has been shown that a peptide derived from the precursor of gonadotropin-releasing hormone may represent the sought-after prolactin-inhibiting factor [33,34], thus demonstrating that pro-sequences of hormones may contain other biologically active peptides. It remains to be seen whether peptide proSS<sub>1-32</sub> plays a regulatory role of its own.

Meanwhile an antiserum has been raised directed against proSS<sub>1-32</sub> (W.E. Schmidt, unpublished) which should facilitate studies of the intracellular processing of the somatostatin precursor and the physiological function of its N-terminal sequence.

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